VISCOSITY VALUES OF PROTOPLASM AS DETERMINED BY MICRODISSECTION[1]

WILLIAM SEIFRIZ

Introduction

The first descriptions of protoplasm, written nearly a century ago, characterize it as "a living jelly." While protoplasm is often of high viscosity, any restricted statement is likely to be misleading, for the viscosity of protoplasm may vary from a consistency slightly more than that of water to that of a firm jelly. From descriptions to be found in current literature it is rather difficult to know of just what degree of viscosity the living substance might be. The difficulty lies in the fact that there has been no careful systematic attempt to ascertain the exact degree of consistency of protoplasm from numerous types of cells and under many different physiological conditions. The following paper represents such an attempt.

Method

The method used has been that of microdissection. The instrument employed in this method is a modification of the Barber pipette holder (1). It consists essentially of two needle holders, each capable of three movements. The holders are fastened to the microscope stage, and the two needles held in them project into a glass moist chamber in which the material to be worked upon is suspended in a hanging drop of water on the under side of a cover slip which forms the roof of the chamber. The needles are of glass and possess exceedingly fine but rigid tips. The technique of the microdissection method is fully described by CHAMBERS (7), to whose article the reader is referred.

The harmful consequences which are likely to result from the practice of holding material in a thin water film, and the importance of this to microdissectionists, make it advisable to direct attention

[1] Botanical Contribution from the Johns Hopkins University, no. 66.

to the frequently damaging effect of this method by presenting some experimental data.

In working with material in a hanging drop it is often very desirable to have the water film of such thinness that the material is held firmly against the cover slip. The surface tension between water and glass thus produced is quite sufficient to hold an active protozoan such as *Euplotes* in a fixed position, or to flatten out marine ova, making them more transparent and dissection easier. If the material thus subjected to surface tension suffers no appreciable distortion, no harmful consequences may result, but eggs of seaweeds and echinoderms are sufficiently pliable to be readily distorted, so that the protoplasm is subjected to an abnormal strain which frequently causes rapid deterioration.

In order to ascertain in a general way the harm done through the flattening of marine ova by a water film, I made a series of observations on the ova of the sea-urchin *Tripneustes*. These eggs were placed in a large and much spread water droplet, which was of greater depth at its center than the diameter of an egg, while toward the periphery the water film gradually thinned to an imperceptible depth. Ova in the center of the droplet were of a normal spherical shape, while those in the peripheral film were much flattened. In half an hour the viscosity of the protoplasm of those eggs in deep water had risen only slightly, while the viscosity of those in the peripheral film had greatly increased. In an hour the spherical ova had risen in viscosity a barely perceptible amount, while the flattened ones had become a firm gel. Furthermore, the distorted eggs tolerated less dissection before showing pronounced disorganization, retained the capacity for healing a wound for a shorter time, and, in a great number of cases, their entire contents dispersed suddenly at the first touch of a needle.

While the use of the surface tension of a thin water film is of value in holding material for microdissection, therefore, the method must be used cautiously. Indeed, the greatest care possible must be taken to keep the material living and normal, and to become familiar with those criteria which assist in ascertaining the exact condition of the living substance. A full appreciation of the extreme irritability of protoplasm is a prerequisite to successful microdissection.

Terminology

Biological terms of uncertain connotation will be considered when the term is first used. I shall discuss briefly a few important words in colloid chemical nomenclature which have no fixed and precise meaning to either chemist or biologist, although they are extensively used by both. THOMAS (**26**) has recently published an account of the nomenclature employed in colloid chemistry, and has in most instances suggested the preferable term to use. I shall follow his terminology strictly, with the possible exception of the words "gel," "jelly," and "coagulum," regarding which THOMAS comes to no definite decision.

In view of the widespread and lax use of the term gel it seems advisable to use the word for the general condition of a colloidal sol when it hardens, whether the hardening be through pectization, that is, coagulation of the sol (the original sense in which gel was used by GRAHAM), or whether through setting (of a gelatine sol, for example), that is, gel includes both the stiffened jelly-like form of a sol, which will redisperse, and the hard amorphous form which will not redisperse. I shall designate gels by stiffening as jellies (which has the support of BECHHOLD **3,** p. 4), and gels by coagulation as coagula.[2] Thus do we also have the corresponding verbs gelate (proposed by TAYLOR **25,** p. 10), to form gels; set, to form jellies; and coagulate, to form coagula.[3]

The employment of three terms where two might suffice may be objectionable in some respects, but it has much to recommend it when protoplasm is the colloid being studied. When protoplasm stiffens it is impossible always to determine definitely whether the mass is a coagulum or a jelly. If the hardening is excessive an undoubted coagulum results, for the living substance is then irreversible, and can be cut up into solid chunks, while, if the

[2] THOMAS (**26,** p. 12) states that BECHHOLD restricts "gel" to the coagula of sols, and terms stiffened sols "jellies." The former statement is not supported by BECHHOLD'S more recent work (**3,** p. 4) where he says: "It seems preferable to me to use the expression "gel" for the general comprehensive phenomenon, and to reserve the word "jelly" for the gelatinization of a hydrophile colloid" (that is, for the gelatinized hydrophile colloid).

[3] HARDY (**12**) employs the last two terms in the same way. "The production of an insoluble gel I shall call "coagulation," that of a soluble gel "stiffening."

increase in consistency is not too great, a firm but resilient and cohesive jelly results. Between these two conditions the exact colloidal state cannot be readily determined. The general term gel is consequently applicable.[4]

Scale of viscosity values

All sciences suffer from a confusion in nomenclature, but perhaps biology more than any other. For example, "viscosity" to the physicist is a specific and accurately measurable property of matter. All fluid substances have their mathematically determined coefficients of viscosity in physics, but to the biologist viscosity has a meaning no more definite than that the substance described has a density somewhere between that of water and of steel. This vagueness, however, is not altogether the biologist's fault.

Protoplasm cannot be collected in sufficient quantity, nor would it remain normal long enough to determine its viscosity, as the physicist would determine that of a liquid, by running it through a viscosimeter; consequently, any attempt to specify the consistency of protoplasm will be more or less influenced by personal opinion. The personal element, however, can be restricted to observations, and not be allowed also to determine the connotation of loose expressions, such as "non-viscous" (an impossible term) and "very viscid sol," which may have quite a different meaning from that conveyed to the reader.

In a previous paper (23) a scale of viscosity was devised so that an expression such as "decidedly viscous" would hold a definite place, and thus convey some idea of approximately how viscous a "decidedly viscous" substance is. I shall retain this

[4] I have heard from the younger colloid chemists that true gels are obtainable only from emulsoids; therefore the coagulation of a suspensoid is not a gel; that is, a gel is not a coagulum; it is a jelly. Some gels (jellies) are reversible and some not. To exclude coagulation as a word descriptive of the process of hardening of emulsions would be altogether too radical a departure in biology, where we constantly speak of the coagulation of blood, of albumin, and like emulsions. I shall, with BECHHOLD, consider as coagulations such processes as cause an irreversible change, whether from emulsions or suspensoids. It would be a great service to biologists, and undoubtedly to colloid chemists themselves, if the usage of gel, jelly, and coagulum were fixed, even though arbitrarily and but tentatively.

scale, but give in addition viscosity values to each degree of consistency. These values are directly referable to definite concentrations of a dispersion (colloidal solution) of gelatine, each concentration, as nearly as could be determined, having the same consistency as that of the protoplasm which has been given a corresponding viscosity value. The gelatine used is a commercial one and readily obtained.[5]

Examination of the gelatine dispersions was done at room temperature (18° C.) in the moist chamber of the microdissection instrument, with needles identical with those employed for the dissection of protoplasm. Table I shows the viscosity values and

TABLE I

Viscosity value*	Descriptive term	Percentage of gelatine†	Substances having an equivalent viscosity‡
1.....	Watery	0.0	Water
2.....	Very liquid	0.05	.....
3.....	Liquid	0.2	.....
4.....	Slightly viscous	0.4	.....
5.....	Rather viscous	0.5	Paraffine oil.....
6.....	Decidedly viscous	0.6	.....
7.....	Very viscous	0.7	Glycerine
8.....	Extremely viscous	0.8	Bread-dough
9.....	Gel	1.0	Vaseline
10.....	Rigid gel	2.0	Firm gelatine

*The abbreviation v.v. will be used for viscosity value.
† A 1 per cent dispersion is a mixture of 1 gm. of gelatine in 99 cc. of water.
‡ All substances were examined at room temperature, 18° C.

corresponding percentages of gelatine which have been established, and these will serve as standards for the viscosity values of protoplasm given in this paper.

Just how accurate percentages of gelatine dispersions are going to prove to be as standards of viscosity is somewhat doubtful, in view of the sensitivity of gelatine to such influences as electrolytes, length of period warmed (**25,** p. 121), etc., which greatly influence its solidification temperature. The percentages of concentration at which gelatine will set, as given in the literature, vary considerably. TAYLOR states that a 10 per cent dispersion of gelatine

[5] Bacto-gelatine, "Difco" standardized, in granular form, with a moisture content less than 4 per cent, made by the Digestive Ferments Company of Detroit, and sold by Arthur H. Thomas Company of Philadelphia.

will set at a temperature of 21° C. This value as a minimum for that temperature must certainly be erroneous. BECHHOLD speaks of a 1 per cent dispersion of gelatine as a semifluid gel (presumably at room temperature), and states that "aqueous" solutions (containing only 1 per cent of water-free gelatine) gelatinize at "ice-box temperature." I find that as low as an 0.8 per cent dispersion (depending on the kind of gelatine used) will gelatinize, that is form a soft jelly, at 18° C. In view of the fact that I have employed a standardized gelatine, it is possible that the values of viscosity given may be fairly accurately verified. I have deemed it advisable to add another column of familiar substances, however, which will help stabilize the scale, and which will also more clearly indicate the exact consistency meant by any given value.

Viscosity as a criterion of the sol and gel states

While a high consistency of an emulsion is almost conclusive evidence of the gel state and a very low consistency evidence of the sol state, yet viscosity alone is not a dependable criterion of the sol and gel conditions. When the viscosity is of an intermediate grade it is of no value as even an approximate indication of the colloidal state. It will be well, therefore, to consider to what extent we are justified in using the terms gel and sol as descriptive of the physical state of protoplasm when viscosity is the only criterion. Although we read that "gels are solid" (**19,** p. 230), and although the word gel connotes to most of us a rather firm though elastic body, it is not necessarily true that all gels are rigid. In fact, this is not the case; a gel may be quite soft. FREUNDLICH, I believe, uses as an indication of the gel state the fact that the substance will support a glass rod placed upright in it. Any criterion of the gel state based on viscosity alone at best can be only approximate. Physical structure and not viscosity determines the colloidal state. We have really no ready means of determining in all instances the actual physical structure of protoplasm, and probably, therefore, should do away with the expressions sol and gel when the degree of viscosity is the only indicator of one state or the other. Where the consistency is very high or very low, however, we can safely characterize the living emulsion

as being respectively in the gel or sol state. It is the intermediate degrees of viscosity of protoplasm of whose physical structure and therefore colloidal state we are totally ignorant. Extremely viscous protoplasm (v.v. 8) is probably a gel, but whether very viscous or decidedly viscous protoplasm is cannot be conclusively stated. This matter may not appear to be a serious one at first, but a brief consideration of the indiscriminate use of the term gel in the literature will show the misinterpretations to which such usage leads.

BAYLISS (2) states: "There is one fact about which there can be no doubt, that is, that protoplasm behaves as a liquid"; while MATHEWS (19) states that protoplasm "is a jelly-like substance or technically a gel." An older investigator (RHUMBLER 22), during a discussion on the structure of protoplasm, says that "protoplasm cannot be a solid substance," while a recent worker (HYMAN 13) says, the fact "that isolated pieces of protoplasm assume the spherical form is not necessarily a proof of its fluid condition," and that "protoplasm is in the gel state." Each of these statements is supported by some experimental facts, but each is true only in part. Protoplasm does not always behave as a liquid (the highly viscous, quiescent protoplasm of bread mold, for example), nor is it always a gel (as for example the very liquid endoplasm of *Euplotes*). KITE (15) has well expressed the exact state of affairs as follows: "Living matter occupies an intermediate position between true solids and true liquids and has many of the properties of both as well as properties peculiar to itself. It belongs to the class of colloids known as emulsoids and exists in either a gel (hydrogel) or a sol (hydrosol) state." Unfortunately, however, KITE then proceeds to use gel as descriptive of protoplasm regardless of viscosity. To be sure, viscosity is not a precise index of physical structure, but with ordinary illumination we have no other criterion by which to judge the exact colloidal state of protoplasm, and a low viscosity is strongly suggestive of a sol, or at least does not suggest a gel state. Such statements as "The living endoplasmic substance is a very dilute and apparently homogeneous gel," and "This structure (the jelly surrounding the egg of *Asterias*) has a low viscosity for a gel and is therefore extremely dilute," are inexact and can

only lead to misinterpretations regarding the true viscosity of protoplasm.

It is very probable that much of the unfavorable criticism of KITE's work is due, not to faulty observations, but to his lax use of the term gel. For example, GARREY (10) claims that the perivitelline space of a fertilized echinoderm egg is filled with a liquid and not with a gel as KITE (according to GARREY) maintains; but who is to decide that the liquid which GARREY sees is not the same kind of substance which KITE saw if gel means one thing to KITE and another thing to GARREY? Sol and gel should be used with great caution in describing the physical state of matter when one is dealing with an emulsion. It is better to give some idea of the viscosity of the substance and let that suggest a possible sol or gel state. It is well to emphasize the fact that protoplasm is not a simple two-phase colloidal system, as one is led to believe from reading much of the literature. On the contrary, it is a multiphase system, emulsion within emulsion.

General viscosity values of protoplasm

The viscosity of protoplasm is not fixed, for it varies in different organisms, in the same organism at different times, and even in different regions of the same organism at the same time. Furthermore, the viscosity of the constituent parts of the protoplasm (the matrix and the protoplasmic inclusions) may differ from the viscosity of the protoplasm as a whole, and from each other. The examination of an inactive Myxomycete plasmodium will frequently reveal a ground substance of very low viscosity, while the mass of protoplasm as a whole is of high consistency. This relation between the viscosity of the constituents of an emulsion and that of the emulsion as a whole is very evident in certain artificial emulsions. For example, in a dispersion of gelatine the viscosity of the medium (water) at 20° C. is 0.012, while the viscosity of the emulsion, of only 2 per cent concentration, is three times as great (0.037).[6] The most striking example of the high consistency of an emulsion as compared with the low viscosity of its dispersion

[6] This reading (from TAYLOR 27) seems surprisingly low, since a 2 per cent concentration of gelatine will ordinarily set into a jelly at a room temperature of 18° C.

medium is the case of castor-oil soap, a 0.1 per cent concentration of which is an almost solid jelly (21).

Thus is it seen that in giving viscosity values it is necessary to distinguish between protoplasm as a whole and its constituent parts, especially the matrix (hyaloplasm). I prefer to use the term matrix rather than hyaloplasm, owing to the confusion which exists in the use of the latter word. Hyaloplasm, as first used by HANSTEIN (11), designated "the homogeneous ground substance" of protoplasm as distinguished from the granules suspended in it. Homogeneity of the ground substance, however, is not definitely established. CHAMBERS (6) and WILSON (27) have employed hyaloplasm to mean the "interalveolar substance." WILSON (29), however, admits the possibility of using the word in the exactly opposite connotation; that is, the ground substance (also termed cell sap, enchylema, hyaloplasm, etc.) is the "alveolar substance" which fills the alveoli. If we accept BÜTSCHLI'S (4) contention that the hyaloplasm (the peripheral granular-free border) of Myxomycetes is not homogeneous, but is of a definite alveolar structure, then this hyaloplasm must be regarded as including both phases of the emulsoid structure; that is, as consisting of interalveolar and intraalveolar substance.

Material

The data upon which the following discussions are based were obtained by a study of a considerable variety of material. Consequently, the conclusions reached may be regarded as rather generally applicable. Since prominent dissimilarities do occur in the properties of widely differing and sometimes of closely related genera, however, it is to be understood that the statements made refer only to the organism under discussion at the time, although many of the general deductions apply to the protoplasm of all the organisms worked upon, if indeed they are not applicable to all living substance.

The following types are the chief ones which were used for this study: the Myxomycetes *Ceratiomyxa*, *Badhamia*, *Arcyria*, *Cribraria*, and *Fuligo;* the rockweed *Fucus;* the fresh water algae *Spirogyra* and *Vaucheria;* the bread molds *Rhizopus* and *Zygorhynchus;* pollen tubes of the blue-flag *Iris versicolor*, of the beach-

pea *Lathyrus maritimus*, and of the dog's-tooth violet *Erythronium revolutum;* the protozoa *Amoeba* and *Euplotes;* the sand-dollar *Echinarachnius;* and the sea-urchin *Tripneustes esculentus*.

The experimental work on these forms was done mostly in the Botanical Laboratory of the Johns Hopkins University. The work on *Fucus*, *Echinarachnius*, and part of that on Myxomycetes and pollen tubes was carried on at the Harpswell Laboratory, South Harpswell, Maine.[7] The work on *Tripneustes* was done at Ocho Rios, Jamaica, B.W.I.[8]

I am greatly indebted to Professor DUNCAN S. JOHNSON for first pointing out to me the possibilities of microdissection as applied to the study of living protoplasm, and for assistance during the progress of this work. To Professor ROBERT CHAMBERS of the Cornell Medical College, New York City, my thanks are due for many suggestions relative to microdissection. I wish also to acknowledge the help received from Dr. CHARLES V. TAYLOR pertaining to the structure and behavior of the protozoan *Euplotes*, and from Dr. HOWARD E. PULLING, then of this university, pertaining to problems in physical chemistry. To Professor WARREN K. LEWIS of the Medical School of this university I am indebted for the loan of the microdissection instrument used in this work.

Myxomycetes

The consistency of Myxomycete protoplasm when in the active vegetative state is liquid (v.v. 3). One is quite likely to be misled by the apparent ease with which a needle traverses protoplasm into believing that the protoplasm is of watery consistency. Superficial observation of streaming protoplasm also leads one to believe that it must be very liquid, while, as a matter of fact, it may be rather viscous, as in bread mold. A good indicator of the degree of viscosity, when the protoplasm is of low consistency, is the distance from the path of a moving needle at which granules are disturbed. The presence of Brownian movement suggests a low consistency.

[7] I am indebted to Director J. S. KINGSLEY for the use of a room at the Harpswell Laboratory.

[8] To FRANK CUNDALL I am greatly obliged for his kindness in placing the facilities of the Institute of Jamaica at my disposal.

The density of the quiescent plasmodium is very high, possessing a maximum viscosity value of 8, and at times approaching the consistency of a gel, but not possessing the firmness of a rigid gel, for the protoplasmic mass of a quiescent Myxomycete is poorly resilient, although quite tough and elastic, and often possessed of a somewhat plastic quality, in this respect closely resembling bread dough. One prominent characteristic of Myxomycete protoplasm is that it is extremely glutinous. This is in striking contrast with marine ova, the protoplasm of which is not noticeably mucilaginous. As a Myxomycete prepares to fruit, the protoplasm increases in viscosity, until it becomes of gel consistency.

Very frequently a tear in a highly viscous, inert plasmodial mass will cause the formation of a rapidly enlarging protrusion. The liquid which flows into and increases the size of such a globule is a granule-free, hyaline substance, to all appearances identical with the peripheral hyaloplasm, but its origin is not peripheral, for this flow of translucent fluid has its source within the protoplasmic mass. Such behavior seems to favor LEYDIG'S conception of the structure of protoplasm, namely, a framework of spongioplasm permeated by a more liquid hyaloplasm (enchylema). By the use of pressure REINKE and RODEWALD (4) obtained 66 per cent of fluid enchylema from the plasmodium of *Aethalium*. The nature of this exuded liquid substance from a plasmodium cannot be stated with certainty. It appears to be the matrix in which the protoplasmic granules are imbedded, or, more accurately, the enchylema (interstitial substance), since the protoplasm is in the gel state and probably of sponge structure.

Amoeba

In many respects *Amoeba* closely resembles the slime molds. Both organisms have periods of motility and non-motility. The former period is characterized by protoplasmic streaming and the formation of pseudopodia, and by a rather liquid condition of the protoplasm; the latter, by protoplasm that is quiescent and more viscous. Both types of organisms are also differentiated into three more or less distinct regions, namely, the inner less viscous

endoplasm, the outer more viscous ectoplasm, and the peripheral highly viscous protoplasmic membrane.[9]

The flowing endosarc of an active *Amoeba* is of slightly viscous consistency (v.v. 4). When quiescent, the endoplasm becomes of a rather or even a decidedly viscous density (v.v. 5 or 6), but seldom higher, never in the living condition attaining a gel consistency (encystment would probably be an exception to this). Brownian movement of particles is generally present and very pronounced throughout the endoplasm of an active *Amoeba*. This suggests a liquid condition. In the quiescent protoplasm of an inactive *Amoeba* the number of particles exhibiting Brownian movement is decidedly less and the amplitude of vibration is reduced, which are evidences of an increased viscosity. The viscosity of the ectosarc is much higher than that of the endosarc, and, as in the latter, varies inversely with activity. The most pronounced decrease in consistency of the ectoplasm occurs in the region immediately concerned in pseudopodium formation, that is, at the tip of an advancing pseudopodium. Here, in a rather restricted center, the ectoplasm becomes quite liquid, which condition, of course, is conducive to the making of a pseudopodium. The liquid condition is temporary and brief. The ectoplasm not directly taking part in amoeboid movement is of very viscous consistency (v.v. 8).

Investigators generally recognize the high viscosity of the ectosarc of *Amoeba*. For example, JENNINGS (14) says that the ectosarc shows "the characteristics of matter in the solid state of aggregation," and HYMAN (13) concludes that the ectoplasm is a gel, "semi-rigid and more or less solidified." The latter, however, although recognizing the possibility of "real fluidity" of the "surface layer," goes too far when assuming that the ectoplasm may attain "extreme solidity." This conclusion is apparently based in part on KITE's (15) statement that "little difficulty is experienced in cutting it (the ectoplasm of *Proteus*) into pieces as small as the limit of microscopical visibility." Here (as in the

[9] Some observers would restrict this differentiation to two regions, not recognizing a distinct protoplasmic membrane.

case of the cytoplasm of the *Asterias* egg which KITE describes as "a quiet translucent gel . . . . which can be cut into small pieces") KITE was either dealing with dead protoplasm, or else the expressions used convey an impression which he himself did not mean. In none of the material worked upon have I been able to "cut" the living protoplasm into small pieces.

There is no doubt that when one is able to cut protoplasm "into pieces as small as the limit of microscopical visibility," the protoplasm is no longer normal. It is not clear just what degree of viscosity KITE wishes to attribute to the ectosarc of *Amoeba*. He states that "this living substance has a moderately high viscosity," but he also says that it is a "quite concentrated gel" (p. 155). KITE'S use of the term gel is very broad. The nearest approach to a firm gel condition of the protoplasm which I have studied is that of the ectosarc of the ciliate *Euplotes* and the quiescent protoplasm of bread mold, but even here the protoplasm possesses considerable plasticity, and, although holding its shape when freed, is of soft rather than solid consistency.

HYMAN credits CHAMBERS with confirming these results of KITE. This is not altogether true. To be sure, CHAMBERS (5) does say that "the external surface of the egg is a gel," and that the surface layer of marine ova is directly comparable with the "rigid ectoplasm" of Protozoa; but he nowhere states that normal living protoplasm can be cut into small pieces. Quite the contrary, he calls attention to the fact that it is the protoplasmic "coagulate" (and a coagulum, as HYMAN points out, is "incompatible with life") that can be "cut into pieces which hold their shape"; and adds that this is likely "to lead one to the erroneous conclusion that the substance of a cell is usually a solid protoplasmic gel." It is true, however, that the ectoplasm of *Amoeba*, when not immediately concerned in pseudopodium formation, is of high consistency, possessing, as HYMAN says, many properties of solids, such as great elasticity, extreme viscosity, and compressibility; and this is sufficient to support the interesting theory (first advanced by MONTGOMERY, according to MCCLENDON 20) that amoeboid movement is "due to alterations of the colloidal state" (13), that is, it is a solation-gelation phenomenon.

Rhizopus

In the bread mold *Rhizopus* (and *Zygorhynchus*) we have, as in Myxomycetes and *Amoeba*, two general states of consistency, changing from one to the other with changes in physiological activity. The protoplasm in the hyphae of bread mold, in the quiescent state, is of very high consistency. It possesses the greatest viscosity of any living plant protoplasm which I have observed by the aid of microdissection.[10] It is of gel consistency, more usually that of a soft gel (v.v. 9), is sticky, quite elastic, and very extensile, closely resembling bread dough in these physical properties. At times it exhibits some resiliency, and may then be characterized as a rigid gel (v.v. 10). KITE (15) has described the living substance of the striped muscle cell of *Necturus* as "the most viscous, elastic, and cohesive of the living gels we have so far considered." The protoplasm of the cells of nerve and muscle tissue is probably of as high a viscosity as any living animal plasma; but it must not be concluded from these observations that this gel consistency is necessarily permanent. Nerve and muscle protoplasm probably exist, just as all the protoplasm so far considered exists, in the sol state at times. Indeed, certain theories of muscle contraction based on "a temporary redistribution of the more fluid portion of the tissue" (LILLIE, 18), and on "coalescence (incipient coagulation) of colloidal particles," which is reversed during the relaxation phase (17), demand a varying viscosity of muscle cells.

Ordinarily, when a filament of bread mold is torn the inactive protoplasm will not flow out, but when pressure is exerted by a needle some distance back from the torn end, the gelled protoplasm can be forced out in the form of a rod, just as one would squeeze oil paint from an artist's tube, and this rod maintains its shape until disturbed.

The streaming protoplasm of *Rhizopus*, as one would expect, is considerably less viscous than the quiescent protoplasm, but it

[10] The viscosity of plant protoplasm which is undergoing long periods of rest, for example, that of seeds, is undoubtedly of even greater concentration, for here the water content is reduced to 20 per cent, while in protoplasm in which pronounced metabolic processes are going on the percentage of water is 80 or more.

is not of as low viscosity as it superficially appears to be, and by no means closely approaches the very liquid state of the endoplasm of an active *Amoeba*. In its most fluid condition it is of at least a rather viscous consistency (v.v. 5), while, when slowly streaming, it may be of very viscous consistency (v.v. 7). All gradations exist between the rather viscous condition when streaming and the gel state when quiescent.

Increase and decrease in viscosity of protoplasm in *Rhizopus* are probably dehydration and hydration phenomena. It is interesting to appreciate the extreme rapidity with which these changes may take place. The streaming protoplasm, by pressure of a needle sufficient to close a hypha, may be made to assume instantly such a consistency that not only does streaming cease, but on subsequent tearing of the filament the emptying of the thread is prevented. Choking of the hypha has caused gelation of the plasma, probably through dehydration. Later, without further disturbance by needles, there is a reversal of the phenomenon. Solation takes place (apparently hydration has set in) and the protoplasm of itself flows out of the torn filament.

Euplotes

In the ciliate *Euplotes* there exists a differentiation between endoplasm and ectoplasm more marked than in any other instance of which I am aware. The endoplasm is very liquid, while the ectoplasm has the firmness of a rigid gel. The former consists of a dilute matrix in which a great variety of inclusions are suspended, from minute protoplasmic particles to whole Protozoa taken in as food; while the latter is free of minute granules (microsomes), and presents a beautiful alveolar structure with the characteristic surface alveolar layer. So far as my limited observations on this protozoan go, the very liquid condition of the endoplasm and the gel state of the ectoplasm seem to be constant. I have observed no change from sol to gel and vice versa, nor any appreciable increase or decrease in viscosity. That there must be some such change at division of the organism (for example, solation of the rigid ectoplasm) seems a physical necessity.

Marine ova

The fully mature, normally discharged eggs of *Fucus* are decidedly viscous (v.v. 6). The unripe eggs are of lower viscosity. The properties and behavior of the ova of *Echinarachnius* and *Tripneustes* are so similar that they can be considered together. The consistency of the mature unfertilized eggs of *Echinarachnius* and *Tripneustes* is a trifle higher than the protoplasm of *Fucus* ova, but barely of very viscous consistency (v.v. 7). KITE has described the cytoplasm of the *Asterias* egg as "a quiet translucent gel." CHAMBERS (5) calls attention to the fact that KITE's paper "is a pioneer one in microdissection research. The observations recorded were necessarily fragmentary." KITE was probably dealing with degenerate gelated protoplasm, or else he fully appreciated the true viscosity of the normal protoplasm of an echinoderm egg and has erroneously described it by his loose use of the term gel. CHAMBERS (6) states that "the interior cytoplasm of a marine egg is a viscous fluid. The viscosity is high enough to prevent any Brownian movement of the inclosed granules." Since the expression "viscous fluid" holds no place in a scale of viscosity, nor is it compared with any other commonly known substance, it is not quite clear just how viscous is the living viscous fluid of a marine egg. The minimum viscosity which the absence of Brownian movement (a criterion used by CHAMBERS) will permit is apparently the viscosity of concentrated laboratory glycerine. EXNER, according to LEHMAN (16), found that "the concentration of ordinary commercial glycerine (specific gravity 1.21) was just enough to put a complete stop to the vibration." A very slight dilution of concentrated glycerine (specific gravity 1.25) is sufficient to permit a noticeable Brownian movement of suspended carmine particles. If Brownian movement is impossible in a very viscous substance such as glycerine, which has a viscosity value of 7, then protoplasm in which no Brownian movement is evident must apparently possess a consistency of about this value. It is such a viscosity value (between 6 and 7) which I attribute to echinoderm ova.

Viscosity of nucleus

CHAMBERS (5) states that the resting nucleus of marine ova "exists in the sol state," and describes how it can be pinched into two droplets which run together on coming into contact. KITE (15) says of the viscosity of the nucleus of the starfish egg: "With the exception of the nucleolus, the nuclear substance is all in the sol state." Of the nucleus of *Amoeba proteus*, however, he says: "The whole of the nuclear substance is a highly rigid and granular gel, the minutest pieces of which show no appreciable change when dissected out in distilled water."

Although one must be very cautious in assuming that all protoplasm is possessed of the same physical properties as that particular protoplasm examined, yet on general principles it would seem that it is hardly likely that the nucleus of an ovum is very liquid (that is, a sol), and that of *Amoeba* a highly rigid gel. My observations on the nucleus of *Amoeba* show that its viscosity is also low, as is the viscosity of nuclei of marine ova (as stated by KITE and CHAMBERS). Results based on the examination of isolated pieces of protoplasm are very uncertain, and that minute pieces of the nuclear substance undergo no change when dissected out into water is quite untenable.

The nucleus of *Amoeba* is rather liquid, but by no means watery, for it possesses a slight degree of viscosity. It is apparently in the sol state. The nucleus when freed from the organism increases in consistency, sometimes slowly and sometimes rapidly. The extremely viscous and in all probability partially degenerate substance of an isolated nucleus is very coherent and elastic, capable of being stretched into fine, barely visible threads. Ultimately the isolated nucleus degenerates into a granular coagulum.

Changes in protoplasmic consistency

While protoplasm may be more or less permanently of a definite viscosity (in such forms as *Euplotes*), yet it does undergo reversible changes. For example, the ectosarc of *Amoeba* is characteristically of a high consistency, yet at the tip of an advancing pseudopodium it becomes temporarily very liquid. Also, in Myxomycetes and bread mold the quiescent protoplasm is very

dense, while the streaming plasma is much more dilute. The change from one state to the other is dependent upon (or at least coincident with) physiological (or physical) activity.

The changes in consistency so far considered have had to do only with the one phenomenon of streaming. There are several other factors which bring on changes in protoplasmic consistency, however, such as development (growth), reproduction, mitosis, and pathological conditions. These changes may be in one direction only, and relatively permanent, as the change from a liquid state to a highly viscous one in the process of fruiting in Myxomycetes; or they may be periodic and reversible. The latter type is exemplified in the changes which accompany streaming. Of the former type the gradual increase in consistency during development of the egg of *Fucus* is an example.

DEVELOPMENTAL CHANGES IN VISCOSITY.—The developmental change in viscosity has been fully described in my former publication (**23**). The protoplasm of young uninucleate oogonia is of liquid consistency (v.v. 3). I think this value is more accurate than the "very liquid" one given in the former publication referred to. Nearly mature oogonia, in which division into 8 eggs is just complete, are of slightly viscous consistency (v.v. 4). As the eggs near maturity they increase to the rather viscous stage (v.v. 5), and the fully mature, normally discharged egg is decidedly viscous (v.v. 6). It is interesting to note that this progressive increase in consistency is coincident with a decrease in physiological activity. The young oogonium with protoplasm of liquid consistency is in a state of active growth, while the decidedly viscous ripe egg is in a more or less quiescent state awaiting fertilization.

REPRODUCTIVE CHANGES IN VISCOSITY.—I have already referred to the increase in viscosity of a Myxomycete plasmodium as it prepares to fruit. The liquid density (v.v. 3) of the active vegetative stage becomes, when inactive, extremely viscous (v.v. 8), and, on preparing to fruit, increases to a gel consistency.

CHANGES IN VISCOSITY DURING MITOSIS.—Division following fertilization in marine ova brings on very decided changes in viscosity. Earlier work on the ova of *Fucus* gave evidence of a marked decrease in consistency of the egg protoplasm within half

an hour after fertilization. Subsequent work on marine animal eggs has shown that the change in viscosity due to fertilization is not so simple a phenomenon as one might think from what can be observed in dissecting the egg of *Fucus*. The protoplasm of the ripe unfertilized egg is of comparatively uniform viscosity. Division, following fertilization, brings on pronounced regional differences in consistency. In the egg these differences are wholly obscured by the dense color of the chloroplasts.

The changes in protoplasmic consistency which are a consequence of fertilization can readily be determined in the dividing echinoderm egg. The following data were obtained principally from dissection of the ova of *Tripneustes*, but were in great part substantiated by subsequent work on the ova of *Echinarachnius*. My observations on the viscosity of the echinoderm egg during mitosis are less detailed than those of CHAMBERS (**6**). In brief, CHAMBERS finds that the sphere (the central transparent area of the aster of the mitotic figure) and the astral rays consist of a clear liquid of very low viscosity, while the surrounding cytoplasm is in the gel state, and that there is a "periodic reversal of the sol to the gel state and vice versa" during mitosis. The following data support, in the main, these findings of CHAMBERS.

The mature unfertilized sea-urchin egg is very viscous (v.v. 7) and comparatively uniform in its viscosity. Following fertilization a change in consistency soon takes place. With the first appearance of the aster there is an increase in viscosity of the peripheral cell cytoplasm. By peripheral protoplasm I refer to a broad outer zone as distinct from an inner core, and not to an ectoplasmic layer, a membrane, or the like. This increase in consistency of the general peripheral protoplasm is from the very viscous to the exceedingly viscous state. With the first appearance of the amphiasters there is a pronounced decrease in viscosity of the central region of the cell, and this condition is maintained throughout the intermediate stages of divisions (from middle prophase to late anaphase). Close examination shows that the dilute protoplasm in the center of the mitotic figure makes up the hyaline area surrounding each pole (the "hyaloplasm-sphere" of WILSON **28**).

The rays of the amphiasters, like the two polar spheres, are apparently also of dilute protoplasm. This thin hyaline substance which makes up the astral rays and polar areas is of liquid consistency (v.v. 3). Although quite dilute it is not watery. In connection with the low viscosity of the hyaline rays it is interesting to recall the theories which have been advanced pertaining to the flow of the substance of which the rays are composed. AUERBACH was probably the first to advance the theory that the rays were currents of a protoplasmic substance. Others, notably FOL, have advocated a similar theory. WILSON (**28**) likewise upholds this theory with the statement that "no one . . . . can, I think, doubt that such a centripetal movement occurs, or that the clear hyaloplasm flows inwards to form the growing hyaloplasm-spheres." STRASBURGER (**24**) states that he, with FOL, looks upon the astral rays as "centripetal currents," to which STRASBURGER ascribes the function of "carrying to the astral body substance which serves as nourishment for the new nucleus."

The protoplasm peripherally located, and also that making up the wedge-shaped protrusions which alternate with the hyaline rays and thus give to the mitotic figure its starlike appearance, is all of high consistency, being very or extremely viscous (v.v. 7 or 8).

A very convincing demonstration of the fluidity of the substance of which the astral spheres and rays are composed was obtained through dissection and previous vital staining with neutral red. The eggs were placed in a very weak stain of neutral red at the time they were fertilized, and allowed to remain in the stain during division, which requires about an hour. The clear liquid substance of the spheres and rays stains a brilliant pink, while the surrounding highly viscous granular plasma takes on little if any stain. A stained ovum in the early anaphase of mitosis was torn until a small globule of protoplasm adjoining the egg had been formed. By pressure against the egg the protoplasm from it could be forced into the globule, thus enlarging the latter, and then by pressure against the globule the protoplasm could be forced back into the egg. The protoplasm which could thus again and again be made to flow from the egg into the globule, and vice versa, was the stained hyaline liquid substance of the astral

spheres, and its fluid condition as contrasted with the extremely viscous consistency of the peripheral protoplasm was very noticeable. It was also strikingly evident that the clear liquid substance did not mix with the granular protoplasm during several minutes of kneading of the egg contents.

The interesting question arises, What is this clear liquid substance which makes up the astral polar areas and rays? WILSON (**27**) refers to it as hyaloplasm (matrix).[11] CHAMBERS (**6**) is noncommital and refers to the contents of the sphere "as the sphere substance or sphere liquid." If the sphere and ray substance is hyaloplasm (matrix), it is very likely a modified, and perhaps greatly modified, form of it, and therefore strictly not hyaloplasm. The unusual circumstances under which it is produced rather suggest that it is at least a modified form of the matrix. This same question arose concerning the identity of the exuded globules of clear substance from Myxomycete plasmodia. Whether the sphere substance is a secretion, which does not seem likely, or an extravasation of one of the many complex phases of the living colloidal system cannot be determined.

With the coming of the telophase of mitosis and the disappearance of the aster, the viscosity of the central protoplasm of the egg rises from the low value of the sphere substance (v.v. 3) to a viscosity value of 6, and with the completion of division we have in each daughter cell of the embryo a general protoplasmic consistency identical with that of the egg before fertilization (v.v. between 7 and 8).

PATHOLOGICAL CHANGES IN VISCOSITY.—The changes in viscosity so far considered have all been of living and normal protoplasm. In determining degrees of viscosity of protoplasm it has been necessary to guard carefully against misinterpretations due to the readiness with which protoplasm alters its consistency as a result of dissection and aging, both of which bring on pathological changes which inevitably result in an increase in viscosity.

[11] "The substance thus flowing inwards I shall for the present designate simply as hyaloplasm (equivalent to the 'cyanoplasm' of MORGAN), and I believe it represents wholly or in part the interalveolar or continuous substance lying between the alveolar spheres"—(WILSON **27**).

Prolonged dissection always ultimately causes an increase in the consistency of protoplasm, unless rapid dissolution first takes place. The rate of increase in viscosity varies greatly in different types of organisms, and even in different individuals of the same type. It is surprising how much dissection protoplasm will often tolerate without showing any increase in viscosity or sign of degeneration, but no protoplasm will endure churning by micro-dissection needles indefinitely. The outcome of such ill treatment may be rapid disintegration or a pronounced increase in viscosity, probably gelation. HYMAN (13) states that "the injury of cutting may completely alter the physical state of protoplasm, probably in the direction of liquefaction." This is not true unless the injury is sufficient to cause complete degeneration, that is, death. Injury not resulting in death always causes an increase in viscosity of the protoplasm. In the advent of death liquefaction does first take place, followed by coagulation.

The injury to an organism undergoing dissection may be general or local; in either case an increase in viscosity results. If the increase in consistency is local, the injured region may be discarded, apparently by the organism, although actually the living organism plays only a passive part, or it may be reabsorbed, by reverting to the sol state. If the increase in viscosity is general and pronounced, death follows.

An *Amoeba* usually shows little change in viscosity over that of the normal quiescent stage as a result of quite some minutes of dissection. Finally, however, either a sudden and pronounced gelation takes place, or, more often, rapid disintegration results. In Myxomycetes the rate of change in consistency due to physical disturbance is slow and gradual. Ultimately, protoplasm subjected to much dissection will always coagulate, unless preceded by rapid dissolution. A coagulum thus formed at death can be cut up into pieces which exhibit none of the properties of the living substance, such as glutinosity, plasticity, elasticity, etc.

Marine ova are subject to the same pathological changes in viscosity as a result of dissection and aging. The increase in consistency from dissection takes place more rapidly than in Myxomycete protoplasm. Here again the degenerate protoplasm ultimately

coagulates (rapid or slow dissolution may follow as well as precede coagulation). The coagulum is coarsely granular in appearance, suggesting a granular precipitate.

Brownian movement of protoplasmic particles

It is rather surprising how little the presence of Brownian movement of particles in really living protoplasm is appreciated. It is usually said that protoplasm, because of its comparatively high viscosity, will not permit Brownian movement of its particles, and that only in vacuoles, whose contents is a dilute sap, is Brownian movement of particles to be observed in a living cell. The classical example of this is the terminal vacuoles of the desmid *Closterium*. It must be borne in mind that I have reference here only to microscopically visible particles, such as make up the granular plasm, and not to ultramicroscopic colloidal particles which are in a constant state of vibration in probably all liquid protoplasm. When workers refer to "the Brownian movement of particles" (2) contained in protoplasm, they do not always explain whether the reference is to microscopic or ultramicroscopic particles. Usually it is apparently the latter. Brownian movement of suspended microscopic particles in protoplasm cannot be seen in by any means all cells or organisms, but it is to be observed in the dilute endoplasm of some ciliates, in the liquid protoplasm of streaming Myxomycetes, and to a striking degree in one of the most studied of organisms, *Amoeba*.

In a quiescent *Amoeba* the number of particles exhibiting Brownian movement is small, and the amplitude of the movement is short. In an active *Amoeba*, the protoplasm of which is more dilute, all particles except the largest droplets exhibit Brownian movement, the motion varying inversely as the size of the particle (4 μ seems to be the maximum size of particles capable of the vibration, according to YOCOM 30), and the amplitude of vibration is relatively large. In the liquid endoplasm of the ciliate infusorian *Euplotes* the suspended particles are frequently in a state of vibration, especially when cyclosis is taking place.

The protoplasm of *Amoeba* in which Brownian movement is to be observed is of rather liquid consistency, and when this proto-

plasm increases in viscosity coincident with a decrease in activity, both the number of particles exhibiting Brownian movement and the amplitude of vibration are lessened. This fact is expressed in the physical law which states that the amplitude of vibration of a particle of a given size is inversely proportional to the viscosity of the dispersion medium (8). This law is further supported by the fact that in highly viscous living protoplasm no Brownian movement is to be seen.

Brownian movement of particles is so characteristic a phenomenon of degenerate protoplasm that I was led to look upon it as an "unfailing criterion of degeneration" (23). Such a conclusion, in view of the presence of Brownian movement in living protoplasm, is not justifiable. It is true, however, that one of the first signs of degeneration in protoplasm, which in the living normal condition shows no Brownian movement, is the instant assumption of a marked oscillating motion of the protoplasmic particles. The surprising thing, however, is that this Brownian movement is actually taking place in an apparently highly viscous mass. Careful dissection will reveal the fact that the degenerate protoplasm has gelated only at the surface, sometimes to an appreciable depth, while the interior of the mass is very dilute. It is in this watery degenerate protoplasm that the suspended particles are in vibration.[12] Death, therefore, has resulted in a liquefaction, probably due to excessive imbibition of the protoplasm. This fact is not generally realized, primarily because the watery condition is only temporary, since ultimately the whole of the protoplasm becomes a rigid coagulum. GAIDUKOV (9), for example, in describing the death changes which take place in the living emulsion, says that "with death of the plasma a coagulation results, which in the case of slow death, is a precipitation, and of sudden death (by fixation), a congealing." I wish again to emphasize the fact that this liquefaction of the protoplasm is a consequence of death, and not of mere injury. Injury, not resulting in death, invariably produces an increase in viscosity. It is not true, therefore, that Brownian movement of microscopic particles in protoplasm ends with death (2). On the contrary, it is

[12] Diffusion of the dilute protoplasm is prevented by rapid gelation at the surface.

frequently set up as one of the consequences of a death phenomenon, that is, temporary liquefaction. When GAIDUKOV states that at death "the motion of the particles ceases," he undoubtedly has reference to ultramicroscopic colloidal particles, and even here this cessation of motion must sometimes be the ultimate and not always the immediate result of death.

The consistency of concentrated laboratory glycerine (specific gravity 1.25) is just high enough to prevent a visible vibration of carmine particles suspended in it. It is not always evident that the viscosity of living protoplasm in which no Brownian movement is to be seen is as high as that of concentrated glycerine. As a general rule, however, it can be stated definitely that protoplasmic particles in a medium of high consistency are not in vibration, while those in very liquid protoplasm are. As a criterion of the viscosity of protoplasm as a whole I do not regard the occurrence or non-occurrence of Brownian movement as very accurate or conclusive.

Summary

1. Protoplasm is a polyphase emulsoid system.

2. Physical structure and not viscosity determines the sol or gel state of an emulsion. Consequently, while protoplasm undoubtedly exists sometimes as a sol and sometimes as a gel, yet sol and gel as descriptive terms of the physical state of protoplasm must be used with great caution when viscosity is the only criterion.

3. The viscosity of protoplasm ranges from a degree slightly more than that of water to the firmness of a fairly rigid gel.

4. While a certain degree of viscosity may characterize the protoplast as a whole, the latter is always more or less divided into regions, whether larger general protoplasmic regions such as ectoplasm and endoplasm, or smaller localized centers of protoplasmic activity such as nucleus and chromatophores, which differ in viscosity from the protoplasm as a whole.

5. Some protoplasmic regions do not noticeably vary in their consistency, but the viscosity of a protoplast as a whole generally varies considerably within a rather wide range.

6. Some of the factors influencing changes in protoplasmic consistency are periodic changes in physiological activity, development, reproduction, mitosis, injury, and death.

7. Streaming protoplasm is less viscous than quiescent protoplasm.

8. Young active protoplasm increases in viscosity as it matures and becomes less active.

9. As a Myxomycete plasmodium prepares to fruit, its consistency becomes very high.

10. During mitosis there are very marked regional changes in viscosity.

11. Physical disturbance usually causes a pronounced increase in viscosity, although the rate of increase varies greatly in different individuals.

12. At death protoplasm frequently becomes temporarily very dilute, probably the result of excessive imbibition. Ultimately the degenerate protoplasm coagulates into a solid granular mass, if rapid dissolution has not preceded coagulation.

JOHNS HOPKINS UNIVERSITY
BALTIMORE, MD.

LITERATURE CITED

1. BARBER, M. A., The pipette method in the isolation of single microorganisms and in the inoculation of substances into living cells. Phil. Jour. Sci. B. 9:307–360. 1914.
2. BAYLISS, W. M., Principles of general physiology. London. 1918.
3. BECHHOLD, H., Colloids in biology and medicine. New York. 1919.
4. BÜTSCHLI, O., Protoplasm and microscopic foams. Transl. London. 1894.
5. Chambers R., ———, Microdissection studies. I. The visible structure of cell protoplasm and death changes. Amer. Jour. Physiol. 43:1–12. 1917.
6. CHAMBERS, R., Microdissection studies. II. The cell aster: a reversible gelation phenomenon. Jour. Exp. Zool. 23:483–504. 1917.
7. ———, The microvivisection method. Biol. Bull. 4:121–126. 1918.
8. FREUNDLICH, H., Kapillarchemie. Leipzig. 1919.
9. GAIDUKOV, N., Dunkelfeldbeleuchtung und Ultramikroskopie in der Biologie und in der Medizin. Jena. 1910.
10. GARREY, W. E., The nature of the fertilization membrane of *Asterias* and *Arbacia* eggs. Biol. Bull. 37:287–293. 1919.
11. HANSTEIN, J., Das Protoplasma als Träger der pflanzlichen und thierischen Lebensverrichtungen. Heidelberg. 1880.
12. HARDY, W. B., Über den Mechanismus der Erstarrung in umkehrbaren Kolloidsystemen. Zeitsch. Phys. Chem. 33:326–343. 1900.

13. HYMAN, L. H., Metabolic gradients in *Amoeba* and their relation to the mechanism of amoeboid movement. Jour. Exp. Zool. **24**:55–99. 1917.
14. JENNINGS, H. S., Contributions to the study of the behavior of lower organisms. Carnegie Inst. Pub. no. 16. 1904.
15. KITE, G. L., Studies on the physical properties of protoplasm. I. The physical properties of protoplasm of certain animal and plant cells. Amer. Jour. Physiol. **32**:146–164. 1913.
16. LEHMAN, O., Molekularphysik. Leipzig. 1888.
17. LILLIE, R. S., The relation between contractility and coagulation of the colloids in the ctenophore swimming-plate. Amer. Jour. Physiol. **16**:117–128. 1908.
18. ———, The physiological significance of the segmented structure of the striated muscle fiber. Science **36**:247–255. 1912.
19. MATHEWS, A. P., Physiological chemistry. New York. 1916.
20. MCCLENDON, J. F., Physical chemistry of vital phenomena. Princeton. 1917.
21. OSTWALD, W., Theoretical and applied colloid chemistry. Transl. New York. 1917.
22. RHUMBLER, L., Versuch einer mechanischen Erklärung der indirekten Zellund Kerntheilung. Arch. Entw.-Mech. Org. **3**:527–623. 1896.
23. SEIFRIZ, W., Observations on the structure of protoplasm by aid of micro-dissection. Biol. Bull. **34**:307–324. 1918.
24. STRASBURGER, E., Zellbildung und Zelltheilung. Jena. 1880.
25. TAYLOR, W. W., The chemistry of colloids. London. 1915.
26. THOMAS, A. W., The nomenclature used in colloid chemistry. Science **36**:10–14. 1918.
27. WILSON, E. B., Experimental studies in cytology. I. A cytological study of artificial parthenogenesis in sea-urchin eggs. Arch. Entw-Mech. Org. **12**:529–596. 1901.
28. ———, Experimental studies in cytology. II. Some phenomena of fertilization and cell-division in etherized eggs; III. The effect on cleavage of artificial obliteration of the first cleavage furrow. Arch. Entw-Mech. Org. **13**:353–395. 1902.
29. ———, The cell in development and inheritance. New York. 1911.
30. YOCOM, H. B., The neuromotor apparatus of *Euplotes patella*. Univ. Calif. Publ. Zool. **18**:337–396. 1918.